normally absent in E. coli membranes. Matrix-assisted laser-desorption/time of flight and radiochem. anal. of the product generated in vitro from the model substrate lipid IVA confirms the selective removal of the 1-phosphate group. These findings show that lpxE is the structural gene for the 1-phosphatase. The availability of lpxE may facilitate the re-engineering of lipid A structures in diverse Gram-neg. bacteria and allow assessment of the role of the 1-phosphatase in R. leguminosarum symbiosis with plants. Possible orthologs of LpxE are present in some intracellular human pathogens, including Francisella tularensis, Brucella melitensis, and Legionella pneumophila.

REFERENCE COUNT: 88 THERE ARE 88 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 2 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2003:500602 CAPLUS

DOCUMENT NUMBER: 139:257119

TITLE: Origin of Lipid A Species Modified with **4**-Amino-**4**-deoxy-L-arabinose in Polymyxin-resistant Mutants of Escherichia coli. An Aminotransferase (ArnB) that Generates UDP-**4**-Amino-**4**-Deoxy-L-Arabinose

AUTHOR(S): Breazeale, Steven D.; Ribeiro, Anthony A.; Raetz, Christian R. H.

CORPORATE SOURCE: Department of Biochemistry, Duke University Medical Center, Durham, NC, 27710, USA

SOURCE: Journal of Biological Chemistry (2003), 278(27), 24731-24739
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB In Escherichia coli and Salmonella typhimurium, addition of the **4**-amino-**4**-deoxy-L-arabinose (L-Ara4N) moiety to the phosphate group(s) of lipid A is required for resistance to polymyxin and cationic antimicrobial peptides. We have proposed previously (Breazeale, S. D., Ribeiro, A. A., and Raetz, C. R. H. (2002) J. Biol. Chemical 277, 2886-2896) a pathway for L-Ara4N biosynthesis that begins with the ArnA-catalyzed C-**4**'' oxidation and C-6'' decarboxylation of UDP-glucuronic acid, followed by the C-**4**'' transamination of the product to generate the novel sugar nucleotide UDP-L-Ara4N. We now show that ArnB (PmrH) encodes the relevant aminotransferase. ArnB was overexpressed using a **T7lac promoter**-driven construct and shown to catalyze the reversible transfer of the amino group from glutamate to the acceptor, uridine 5'-(β-L-threo-pentapyranosyl- **4**''-ulose diphosphate), the intermediate that is synthesized by ArnA from UDP-glucuronic acid. A 1.7-mg sample of the putative UDP-L-Ara4N product generated in vitro was purified by ion exchange chromatog., and its structure was confirmed by 1H and 13C NMR spectroscopy. ArnB, which is a cytoplasmic protein, was purified to homogeneity from an overproducing strain of E. coli and shown to contain a pyridoxal phosphate cofactor, as judged by UV/visible spectrophotometry. The pyridoxal phosphate was converted to the pyridoxamine form in the presence of excess glutamate. A simple quant. radiochem. assay was developed for ArnB, which can be used to assay the enzyme either in the forward or the reverse direction. The enzyme is highly selective for glutamate as the amine donor, but the equilibrium constant in the direction of UDP-L-Ara4N formation is unfavorable (.apprx.0.1). ArnB is a member of a very large family of aminotransferases, but closely related ArnB orthologs are present only in those bacteria capable of synthesizing lipid A species modified with the L-Ara4N moiety.

REFERENCE COUNT: 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 3 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:323278 CAPLUS
DOCUMENT NUMBER: 139:175639
TITLE: Relaxed Sugar Donor Selectivity of a Sinorhizobium meliloti Ortholog of the Rhizobium leguminosarum Mannosyl Transferase LpcC. Role of the lipopolysaccharide core in symbiosis of Rhizobiaceae with plants
AUTHOR(S): Kanipes, Margaret I.; Kalb, Suzanne R.; Cotter, Robert J.; Hozbor, Daniela F.; Lagares, Antonio; Raetz, Christian R. H.
CORPORATE SOURCE: Department of Biochemistry, Duke University Medical Center, Durham, NC, 27710, USA
SOURCE: Journal of Biological Chemistry (2003), 278(18), 16365-16371
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The lpcC gene of Rhizobium leguminosarum and the lpsB gene of Sinorhizobium meliloti encode protein orthologs that are 58% identical over their entire lengths of about 350 amino acid residues. LpcC and LpsB are required for symbiosis with pea and Medicago plants, resp. S. meliloti lpsB complements a mutant of R. leguminosarum defective in lpcC, but the converse does not occur. LpcC encodes a highly selective mannosyl transferase that utilizes GDP-mannose to glycosylate the inner 3-deoxy-D-manno-octulosonic acid (Kdo) residue of the lipopolysaccharide precursor Kdo2-lipid IVA. We now demonstrate that LpsB can also efficiently mannosylate the same acceptor substrate as does LpcC. Unexpectedly, however, the sugar nucleotide selectivity of LpsB is greatly relaxed compared with that of LpcC. Membranes of the wild-type S. meliloti strain 2011 catalyze the glycosylation of Kdo2-[4'-32P]lipid IVA at comparable rates using a diverse set of sugar nucleotides, including GDP-mannose, ADP-mannose, UDP-glucose, and ADP-glucose. This complex pattern of glycosylation is due entirely to LpsB, since membranes of the S. meliloti lpsB mutant 6963 do not glycosylate Kdo2-[4'-32P]lipid IVA in the presence of any of these sugar nucleotides. Expression of lpsB in E. coli using a **T7lac promoter**-driven construct results in the appearance of similar multiple glycosyl transferase activities seen in S. meliloti 2011 membranes. Constructs expressing lpcC display only mannosyl transferase activity. We conclude that LpsB, despite its high degree of similarity to LpcC, is a much more versatile glycosyltransferase, probably accounting for the inability of lpcC to complement S. meliloti lpsB mutants. Our findings have important implications for the regulation of core glycosylation in S. meliloti and other bacteria containing LpcC orthologs.
REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 4 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:226539 CAPLUS
DOCUMENT NUMBER: 131:68807
TITLE: A new series of pET-derived vectors for high efficiency expression of Pseudomonas exotoxin-based fusion proteins
AUTHOR(S): Matthey, Barbel; Engert, Andreas; Klimka, Alexander; Diehl, Volker; Barth, Stefan
CORPORATE SOURCE: Laboratory of Immunotherapy, Dep. I of Internal Medicine, University Hospital of Cologne, Cologne, 50931, Germany
SOURCE: Gene (1999), 229(1-2), 145-153
CODEN: GENED6; ISSN: 0378-1119

PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Recombinant immunotoxins (rITs) are highly specific anti-tumor agents composed of monoclonal antibody fragments or other specific carriers coupled to plant or bacterial toxins. A major problem in the purification of rITs is the low periplasmic yield in currently available expression systems. Thus, the aim of this study was the development of a new bacterial expression system for high-level production of rITs. We constructed a series of pET-based vectors for pelB-directed periplasmic secretion or cytoplasmic production under the control of the **T7lac promoter**. Expression in Escherichia coli BL21 (DE3)pLysS allowed a tightly regulated iso-Pr β-d-thiogalactopyranoside (IPTG) induction of protein synthesis. An enterokinase-cleavable poly-histidine cluster was introduced into this setup for purification by affinity chromatog. A major modification resulted from the insertion of a specifically designed multiple cloning site. It contains only rare restriction enzyme recognition sites used for cloning of Ig variable region genes, as well as unique SfiI and NotI restriction sites for directed insertion of single-chain variable fragments (scFv) available from established bacteriophage systems. For this purpose, we deleted two naturally occurring internal SfiI consensus sites in a deletion mutant of Pseudomonas aeruginosa exotoxin A (ETA'). Each single structural element of the new vector (promoter, leader sequence, purification tag, scFv sequence, selectable marker, and toxin gene) was flanked by unique restriction sites allowing simple directional substitution. The fidelity of IPTG induction and high-level expression were demonstrated using an anti-CD30 scFv (Ki-4) fused to ETA'. These data confirm a bacterial vector system especially designed for efficient periplasmic expression of ETA'-based fusion toxins.

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 5 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1997:509895 CAPLUS
DOCUMENT NUMBER: 127:92011
TITLE: Expression of an Aspergillus niger Phytase (phyA) in Escherichia coli
AUTHOR(S): Phillippy, Brian Q.; Mullaney, Edward J.
CORPORATE SOURCE: Southern Regional Research Center Agricultural Research Service, U.S. Department of Agriculture, New Orleans, LA, 70124, USA
SOURCE: Journal of Agricultural and Food Chemistry (1997), 45(8), 3337-3342
CODEN: JAFCAU; ISSN: 0021-8561
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The gene (phyA) for the Aspergillus niger phytase with optima at pH 5.5 and 2.2 was expressed in Escherichia coli under the control of the **T7lac promoter**. A 56 kDa fusion protein comprised of phytase linked to an S-tag leader peptide accumulated in inclusion bodies at 30°. The yield of unglycosylated recombinant phytase purified from 50 mL cultures by anion exchange chromatog. of solubilized inclusion body protein was 10 mg. The refolded enzyme had an activity of 1.5 μmol mg-1 min-1 at 37°, but most of the protein was in the form of inactive aggregates. Recombinant phytase displayed a single pH optimum at pH 5.1, was irreversibly denatured at pH 2.0 and was not active above 55°. As with A. niger phytase, the initial breakdown product observed was inositol 1,2,4,5,6-pentakis(phosphate). Km values for the hydrolysis of inositol hexakis(phosphate) and p-nitrophenylphosphate were 96 μM and 2.0 mM, resp., at pH 4.5.

L3 ANSWER 6 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1996:98228 CAPLUS
DOCUMENT NUMBER: 124:224535
TITLE: Galactofuranose biosynthesis in Escherichia coli K-12: identification and cloning of UDP-galactopyranose mutase
AUTHOR(S): Nassau, Pam M.; Martin, Stephen L.; Brown, Robin E.; Weston, Anthony; Monsey, David; McNeil, Michael R.; Duncan, Kenneth
CORPORATE SOURCE: Glaxo Wellcome Medicines Research Center, Hertfordshire, SG1 2NY, UK
SOURCE: Journal of Bacteriology (1996), 178(4), 1047-52
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We have cloned two open reading frames (orf6 and orf8) from the Escherichia coli K-12 rfb region. The genes were expressed in E. coli under control of the **T7lac promoter**, producing large quantities of recombinant protein, most of which accumulated in insol. inclusion bodies. Sufficient soluble protein was obtained, however, for use in a radiometric assay designed to detect UDP-galactopyranose mutase activity (the conversion of UDP-galactopyranose to UDP-galactofuranose). The assay is based upon high-pressure liquid chromatog. separation of sugar phosphates released from both forms of UDP-galactose by phosphodiesterase treatment. The crude orf6 gene product converted UDP-[α-D-U-14C]-galactopyranose to a product which upon phosphodiesterase treatment gave a compound with a retention time identical to that of synthetic α-galactofuranose-1-phosphate. No mutase activity was detected in exts. from cells lacking the orf6 expression plasmid or from orf8-expressing cells. The orf6 gene product was purified by anion-exchange chromatog. and hydrophobic interaction chromatog. Both the crude extract and the purified protein converted 6 to 9% of the UDP-galactopyranose to the furanose form. The enzyme was also shown to catalyze the reverse reaction; in this case an approx. 86% furanose-to-pyranose conversion was observed These observations strongly suggest that orf6 encodes UDP-galactopyranose mutase (EC 5.**4**.99.9), and we propose that the gene be designated glf accordingly. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of purified UDP-galactopyranose mutase revealed one major band, and anal. by electrospray mass spectrometry indicated a single major species with a mol. weight of 42,960 ± 8, in accordance with that calculated for the Glf protein. N-terminal sequencing revealed that the first 15 amino acids of the recombinant protein corresponded to those expected from the published sequence. UV-visible spectra of purified recombinant enzyme indicated that the protein contains a flavin cofactor, which we have identified as FAD.

L3 ANSWER 7 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1995:492945 CAPLUS
DOCUMENT NUMBER: 122:263611
TITLE: Comparison of the expression of native and mutant bovine annexin IV in Escherichia coli using four different expression systems
AUTHOR(S): Nelson, Michael R.; Creutz, Carl E.
CORPORATE SOURCE: Dep. Pharmacol., Univ. Virginia, Charlottesville, VA, 22908, USA
SOURCE: Protein Expression and Purification (1995), 6(2), 132-40
CODEN: PEXPEJ; ISSN: 1046-5928
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Bovine annexin IV, a Ca2+-dependent, membrane-binding protein, was expressed in E. coli using **4** different prokaryotic expression

vector systems. An annexin IV cDNA was mutated in the 5' noncoding region to introduce an NcoI restriction site at the translation initiation site. The coding sequence was then excised and ligated into the expression vectors: pKK233-2 (which uses a hybrid trc promoter), pFOG405 (which uses the alkaline phosphatase promoter and generates a fusion protein with the alkaline phosphatase signal sequence that targets the protein for secretion), pOTSNco12 (which provides temperature-sensitive expression from the λ phage promoter), and pET11d (which uses the **T7lac promoter** and a protease-deficient host). Expression of wild type and mutant annexin IV in the various systems was compared. Differences in level of expression, formation of inclusion bodies, and yield of purified protein were observed The pET11d system was the most effective expression system for annexin IV and various annexin IV mutant constructs, providing the highest yield of functional protein from the soluble fraction of cell lysates. Bovine chromaffin granule binding and aggregating activities of recombinant annexin IV were virtually indistinguishable from those of bovine annexin IV isolated from liver tissue. Truncation constructs containing 1, 2, or 3 of the **4** conserved 70-amino-acid domains of native annexin IV were successfully created and expressed in E. coli, but the recombinant proteins were generally insol. The pET11d annexin constructs containing point mutations in residues involved in binding Ca produced soluble protein at levels comparable to those of constructs expressing wild type protein.

L3 ANSWER 8 OF 16 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2004:164425 BIOSIS
DOCUMENT NUMBER: PREV200400168185
TITLE: Expression of ornithine decarboxylase of Coccidioides immitis in three Escherichia coli strains carrying the lambda DE3 lysogen and an E. coli EWH319 strain odc- null mutant.
AUTHOR(S): Pantoja-Hernandez, Miguel Angel; Munoz-Sanchez, Claudia Ivonne; Guevara-Gonzalez, Ramon Gerardo; Botello-Alvarez, Enrique; Gonzalez-Chavira, Mario Martin; Torres-Pacheco, Irineo; Guevara-Olvera, Lorenzo [Reprint Author]
CORPORATE SOURCE: Departamento de Ingenieraa Bioquimica, Instituto Tecnologico de Celaya, Ave. Tecnologico y A. Garcia-Cubas, S/N, Colonia FOVISSSTE, Apartado Postal 57, Celaya, GTO, Mexico
lorenzogo@yahoo.com
SOURCE: Biotechnology Letters, (January 2004) Vol. 26, No. 1, pp. 75-78. print.
CODEN: BILED3. ISSN: 0141-5492.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Mar 2004
Last Updated on STN: 24 Mar 2004

AB Ornithine decarboxylase from respiratory fungal pathogen, Coccidioides immitis, cloned in the pETCiODC plasmid under control of **T7lac promoter**, was produced in E. coli BL21(DE3), BL21(DE3)pLysS, BLR(DE3) and EWH319 transformant strains. E. coli BL21(DE3)pLysS-pETCiODC expressed the highest specific activity of ODC, suggesting that this strain could be successfully used for protein structure and drug testing studies.

L3 ANSWER 9 OF 16 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2003:566269 BIOSIS
DOCUMENT NUMBER: PREV200300568168
TITLE: Expression cloning and biochemical characterization of a Rhizobium leguminosarum lipid A 1-phosphatase.
AUTHOR(S): Karbarz, Mark J.; Kalb, Suzanne R.; Cotter, Robert J.;

Raetz, Christian R. H. [Reprint Author]
CORPORATE SOURCE: Dept. of Biochemistry, Duke University Medical Center, P.O. Box 3711, Durham, NC, 27710, USA
raetz@biochem.duke.edu
SOURCE: Journal of Biological Chemistry, (October 10 2003) Vol. 278, No. 41, pp. 39269-39279. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 3 Dec 2003
Last Updated on STN: 3 Dec 2003

AB Lipid A of Rhizobium leguminosarum, a nitrogen-fixing plant endosymbiont, displays several significant structural differences when compared with Escherichia coli. An especially striking feature of R. leguminosarum lipid A is that it lacks both the 1- and **4**'-phosphate groups. Distinct lipid A phosphatases that attack either the 1 or the **4**' positions have previously been identified in extracts of R. leguminosarum and Rhizobium etli but not Sinorhizobium meliloti or E. coli. Here we describe the identification of a hybrid cosmid (pMJK-1) containing a 25-kb R. leguminosarum 3841 DNA insert that directs the overexpression of the lipid A 1-phosphatase. Transfer of pMJK-1 into S. meliloti 1021 results in heterologous expression of 1-phosphatase activity, which is normally absent in extracts of strain 1021, and confers resistance to polymyxin. Sequencing of a 7-kb DNA fragment derived from the insert of pMJK-1 revealed the presence of a lipid phosphatase ortholog (designated LpxE). Expression of lpxE in E. coli behind the **T7lac promoter** results in the appearance of robust 1-phosphatase activity, which is normally absent in E. coli membranes. Matrix-assisted laser-desorption/time of flight and radiochemical analysis of the product generated in vitro from the model substrate lipid IVA confirms the selective removal of the 1-phosphate group. These findings show that lpxE is the structural gene for the 1-phosphatase. The availability of lpxE may facilitate the re-engineering of lipid A structures in diverse Gram-negative bacteria and allow assessment of the role of the 1-phosphatase in R. leguminosarum symbiosis with plants. Possible orthologs of LpxE are present in some intracellular human pathogens, including Francisella tularensis, Brucella melitensis, and Legionella pneumophila.

L3 ANSWER 10 OF 16 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2003:396009 BIOSIS
DOCUMENT NUMBER: PREV200300396009
TITLE: Origin of lipid A species modified with **4**-amino-**4**-deoxy-L-arabinose in polymyxin-resistant mutants of Escherichia coli. An aminotransferase (ArnB) that generates UDP-**4**-amino-**4**-deoxy-L-arabinose.
AUTHOR(S): Breazeale, Steven D.; Ribeiro, Anthony A.; Raetz, Christian R. H. [Reprint Author]
CORPORATE SOURCE: Department of Biochemistry, Duke University Medical Center, Durham, NC, 27710, USA
raetz@biochem.duke.edu
SOURCE: Journal of Biological Chemistry, (July 4 2003) Vol. 278, No. 27, pp. 24731-24739. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Aug 2003
Last Updated on STN: 27 Aug 2003

AB In Escherichia coli and Salmonella typhimurium, addition of the **4**-amino-**4**-deoxy-L-arabinose (L-Ara4N) moiety to the phosphate group(s) of lipid A is required for resistance to polymyxin and cationic antimicrobial peptides. We have proposed previously (Breazeale, S. D.,

Ribeiro, A. A., and Raetz, C. R. H. (2002) J. Biol. Chemical 277, 2886-2896) a pathway for L-Ara4N biosynthesis that begins with the ArnA-catalyzed C-4" oxidation and C-6" decarboxylation of UDP-glucuronic acid, followed by the C-4" transamination of the product to generate the novel sugar nucleotide UDP-L-Ara4N. We now show that ArnB (PmrH) encodes the relevant aminotransferase. ArnB was overexpressed using a **T7lac promoter**-driven construct and shown to catalyze the reversible transfer of the amino group from glutamate to the acceptor, uridine 5'-(beta-L-threo-pentapyranosyl-4"-ulose diphosphate), the intermediate that is synthesized by ArnA from UDP-glucuronic acid. A 1.7-mg sample of the putative UDP-L-Ara4N product generated in vitro was purified by ion exchange chromatography, and its structure was confirmed by 1H and 13C NMR spectroscopy. ArnB, which is a cytoplasmic protein, was purified to homogeneity from an overproducing strain of E. coli and shown to contain a pyridoxal phosphate cofactor, as judged by ultraviolet/visible spectrophotometry. The pyridoxal phosphate was converted to the pyridoxamine form in the presence of excess glutamate. A simple quantitative radiochemical assay was developed for ArnB, which can be used to assay the enzyme either in the forward or the reverse direction. The enzyme is highly selective for glutamate as the amine donor, but the equilibrium constant in the direction of UDP-L-Ara4N formation is unfavorable (apprx0.1). ArnB is a member of a very large family of aminotransferases, but closely related ArnB orthologs are present only in those bacteria capable of synthesizing Hpid A species modified with the L-Ara4N moiety.

L3 ANSWER 11 OF 16 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2003:289851 BIOSIS
DOCUMENT NUMBER: PREV200300289851
TITLE: Relaxed sugar donor selectivity of a Sinorhizobium meliloti ortholog of the Rhizobium leguminosarum mannosyl transferase LpcC. Role of the lipopolysaccharide core in symbiosis of Rhizobiaceae with plants.
AUTHOR(S): Kanipes, Margaret I.; Kalb, Suzanne R.; Cotter, Robert J.; Hozbor, Daniela F.; Lagares, Antonio; Raetz, Christian R. H. [Reprint Author]
CORPORATE SOURCE: Department of Biochemistry, Duke University Medical Center, Durham, NC, 27710, USA
raetz@biochem.duke.edu
SOURCE: Journal of Biological Chemistry, (May 2 2003) Vol. 278, No. 18, pp. 16365-16371. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 19 Jun 2003
Last Updated on STN: 19 Jun 2003

AB The lpcC gene of Rhizobium leguminosarum and the lpsB gene of Sinorhizobium meliloti encode protein orthologs that are 58% identical over their entire lengths of about 350 amino acid residues. LpcC and LpsB are required for symbiosis with pea and Medicago plants, respectively. S. meliloti lpsB complements a mutant of R. leguminosarum defective in lpcC, but the converse does not occur. LpcC encodes a highly selective mannosyl transferase that utilizes GDP-mannose to glycosylate the inner 3-deoxy-D-manno-octulosonic acid (Kdo) residue of the lipopolysaccharide precursor Kdo2-lipid IVA. We now demonstrate that LpsB can also efficiently mannosylate the same acceptor substrate as does LpcC. Unexpectedly, however, the sugar nucleotide selectivity of LpsB is greatly relaxed compared with that of LpcC. Membranes of the wild-type S. meliloti strain 2011 catalyze the glycosylation of Kdo2-(**4**'-32P)lipid IVA at comparable rates using a diverse set of sugar nucleotides, including GDP-mannose, ADP-mannose, UDP-glucose, and ADP-glucose. This complex pattern of glycosylation is due entirely to

LpsB, since membranes of the S. meliloti lpsB mutant 6963 do not glycosylate Kdo2-(4'-32P)lipid IVA in the presence of any of these sugar nucleotides. Expression of lpsB in E. coli using a **T7lac promoter**-driven construct results in the appearance of similar multiple glycosyl transferase activities seen in S. meliloti 2011 membranes. Constructs expressing lpcC display only mannosyl transferase activity. We conclude that LpsB, despite its high degree of similarity to LpcC, is a much more versatile glycosyltransferase, probably accounting for the inability of lpcC to complement S. meliloti lpsB mutants. Our findings have important implications for the regulation of core glycosylation in S. meliloti and other bacteria containing LpcC orthologs.

L3 ANSWER 12 OF 16 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2003:194922 BIOSIS
DOCUMENT NUMBER: PREV200300194922
TITLE: Cloning, analysis, and expression of the gene for thermostable polyphosphate kinase of Thermus caldophilus GK24 and properties of the recombinant enzyme.
AUTHOR(S): Hoe Hyang-Sook; Lee, Sung-Kyoung; Lee, Dae-Sil; Kwon, Suk-Tae [Reprint Author]
CORPORATE SOURCE: Department of Genetic Engineering, Sungkyunkwan University, Suwon, 440-746, South Korea
stkwon@yurim.skku.ac.kr
SOURCE: Journal of Microbiology and Biotechnology, (February 2003) Vol. 13, No. 1, pp. 139-145. print.
ISSN: 1017-7825.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Apr 2003
Last Updated on STN: 16 Apr 2003

AB The gene encoding Thermus caldophilus GK24 polyphosphate kinase (Tca PPK) was cloned and sequenced. The gene contains an open reading frame encoding 608 amino acids with a calculated molecular mass of 69,850 Da. The deduced amino acid sequence of Tca PPK showed a 40% homology to Escherichia coli PPK, and 39% to Klebsiella aerogenes PPK. The Tca ppk gene was expressed under the control of the **T7lac promoter** on pET-22b(+) in E. coli and its enzyme was purified about 70-fold with 36% yield, following heating and HiTrap chelating HP column chromatography. The native enzyme was found to have an approximate molecular mass of 580,000 Da and consisted of eight subunits. The optimum pH and temperature of the enzyme were 5.5 and 70degreeC, respectively. A divalent cation was required for the enzyme activity, with Mg2+ being the most effective.

L3 ANSWER 13 OF 16 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 1999:236621 BIOSIS
DOCUMENT NUMBER: PREV199900236621
TITLE: A new series of pET-derived vectors for high efficiency expression of Pseudomonas exotoxin-based fusion proteins.
AUTHOR(S): Matthey, Baerbel; Engert, Andreas; Klimka, Alexander; Diehl, Volker; Barth, Stefan [Reprint author]
CORPORATE SOURCE: Department I of Internal Medicine, Laboratory of Immunotherapy, University Hospital of Cologne, Joseph-Stelzmann-Str. 9, 50931, Cologne, Germany
SOURCE: Gene (Amsterdam), (March 18, 1999) Vol. 229, No. 1-2, pp. 145-153. print.
CODEN: GENED6. ISSN: 0378-1119.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 17 Jun 1999
Last Updated on STN: 17 Jun 1999

AB Recombinant immunotoxins (rITs) are highly specific anti-tumor agents composed of monoclonal antibody fragments or other specific carriers coupled to plant or bacterial toxins. A major problem in the purification of rITs is the low periplasmic yield in currently available expression systems. Thus, the aim of this study was the development of a new bacterial expression system for high-level production of rITs. We constructed a series of pET-based vectors for pelB-directed periplasmic secretion or cytoplasmic production under the control of the **T7lac promoter**. Expression in Escherichia coli BL21 (DE3)pLysS allowed a tightly regulated isopropyl beta-d-thiogalactopyranoside (IPTG) induction of protein synthesis. An enterokinase-cleavable poly-histidine cluster was introduced into this setup for purification by affinity chromatography. A major modification resulted from the insertion of a specifically designed multiple cloning site. It contains only rare restriction enzyme recognition sites used for cloning of immunoglobulin variable region genes, as well as unique SfiI and NotI restriction sites for directed insertion of single-chain variable fragments (scFv) available from established bacteriophage systems. For this purpose, we deleted two naturally occurring internal SfiI consensus sites in a deletion mutant of Pseudomonas aeruginosa exotoxin A (ETA'). Each single structural element of the new vector (promoter, leader sequence, purification tag, scFv sequence, selectable marker, and toxin gene) was flanked by unique restriction sites allowing simple directional substitution. The fidelity of IPTG induction and high-level expression were demonstrated using an anti-CD30 scFv (Ki-**4**) fused to ETA'. These data confirm a bacterial vector system especially designed for efficient periplasmic expression of ETA'-based fusion toxins.

L3 ANSWER 14 OF 16 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 1997:437550 BIOSIS
DOCUMENT NUMBER: PREV199799736753
TITLE: Expression of an Aspergillus niger phytase (phyA) in Escherichia coli.
AUTHOR(S): Phillippy, Brian Q. [Reprint author]; Mullaney, Edward J.
CORPORATE SOURCE: Southern Regional Res. Cent., Agricultural Res. Service, U.S. Dep. Agriculture, New Orleans, LA 70124, USA
SOURCE: Journal of Agricultural and Food Chemistry, (1997) Vol. 45, No. 8, pp. 3337-3342.
CODEN: JAFCAU. ISSN: 0021-8561.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Oct 1997
Last Updated on STN: 21 Nov 1997

AB The gene (phyA) for the Aspergillus niger phytase with optima at pH 5.5 and 2.2 was expressed in Escherichia coli under the control of the **T7lac promoter**. A 56 kDa fusion protein comprised of phytase linked to an S-Tag leader peptide accumulated in inclusion bodies at 30 degree C. The yield of unglycosylated recombinant phytase purified from 50 mL cultures by anion exchange chromatography of solubilized inclusion body protein was 10 mg. The refolded enzyme had an activity of 1.5 mu-mol mg-1 min-1 at 37 degree , but most of the protein was in the form of inactive aggregates. Recombinant phytase displayed a single pH optimum at pH 5.1, was irreversibly denatured at pH 2.0 and was not active above 55 degree C. As with A. niger phytase, the initial breakdown product observed was inositol 1,2,**4**,5,6-pentakis(phosphate). K-m values for the hydrolysis of inositol hexakis(phosphate) and p-nitrophenylphosphate were 96 mu-M and 2.0 mM, respectively, at pH **4**.5.

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ACCESSION NUMBER: 1997:71285 BIOSIS
DOCUMENT NUMBER: PREV199799370488

TITLE: High level expression of Ricinus communis casbene synthase in Escherichia coli and characterization of the recombinant enzyme.
AUTHOR(S): Hill, Alison M.; Cane, David E. [Reprint author]; Mau, Christopher J. D.; West, Charles A.
CORPORATE SOURCE: Dep. Chem., Box H, Brown Univ., Providence, RI 02912, USA
SOURCE: Archives of Biochemistry and Biophysics, (1996) Vol. 336, No. 2, pp. 283-289.
CODEN: ABBIA4. ISSN: 0003-9861.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 11 Feb 1997
Last Updated on STN: 25 Mar 1997

AB Casbene synthase catalyzes the cyclization of geranylgeranyl diphosphate (2) to casbene (1), a diterpene phytoalexin with antibacterial and antifungal activity that is produced by seedlings of castor bean (Ricinus communis L.) in response to fungal attack. We report the high-level expression of casbene synthase cDNA in Escherichia coli as insoluble inclusion bodies, the solubilization and refolding of active casbene synthase, and the kinetic and product analysis of the recombinant enzyme. To overcome problems apparently associated with the presence in the casbene synthase gene of rare Arg codons, as well as the intrinsic antibacterial activity of casbene itself, the casbene synthase gene was expressed in an E. coli host harboring the pSM102 vector that encodes the dnaY gene for tArg-(AGA/G), using an expression vector, pET-21d(+), carrying the tightly controlled **T7lac promoter**.

L3 ANSWER 16 OF 16 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1996:129548 BIOSIS
DOCUMENT NUMBER: PREV199698701683
TITLE: Galactofuranose biosynthesis in Escherichia coli K-12: Identification and cloning of UDP-galactopyranose mutase.
AUTHOR(S): Nassau, Pam M.; Martin, Stephen L.; Brown, Robin E.; Weston, Anthony; Monsey, David; McNeil, Michael R.; Duncan, Kenneth [Reprint author]
CORPORATE SOURCE: Glaxo Wellcome Med. Res. Cent., Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, UK
SOURCE: Journal of Bacteriology, (1996) Vol. 178, No. 4, pp. 1047-1052.
CODEN: JOBAAY. ISSN: 0021-9193.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Mar 1996
Last Updated on STN: 2 May 1996

AB We have cloned two open reading frames (orf6 and orf8) from the Escherichia coli K-12 rfb region. The genes were expressed in E. coli under control of the **T7lac promoter**, producing large quantities of recombinant protein, most of which accumulated in insoluble inclusion bodies. Sufficient soluble protein was obtained, however, for use in a radiometric assay designed to detect UDP-galactopyranose mutase activity (the conversion of UDP-galactopyranose to UDP-galactofuranose). The assay is based upon high-pressure liquid chromatography separation of sugar phosphates released from both forms of UDP-galactose by phosphodiesterase treatment. The crude orf6 gene product converted UDP-(alpha-D-U-14C)-galactopyranose to a product which upon phosphodiesterase treatment gave a compound with a retention time identical to that of synthetic alpha-galactofuranose-1-phosphate. No mutase activity was detected in extracts from cells lacking the orf6 expression plasmid or from orf8-expressing cells. The orf6 gene product was purified by anion-exchange chromatography and hydrophobic interaction chromatography. Both the crude extract and the purified protein converted 6 to 9% of the UDP-galactopyranose to the furanose form. The enzyme was also shown to catalyze the reverse reaction; in this case an approximately

86% furanose-to-pyranose conversion was observed. These observations strongly suggest that orf6 encodes UDP-galactopyranose mutase (EC 5.4.99.9), and we propose that the gene be designated glf accordingly. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified UDP-galactopyranose mutase revealed one major band, and analysis by electrospray mass spectrometry indicated a single major species with a molecular weight of 42,960 +- 8, in accordance with that calculated for the Glf protein. N-terminal sequencing revealed that the first 15 amino acids of the recombinant protein corresponded to those expected from the published sequence. UV-visible spectra of purified recombinant enzyme indicated that the protein contains a flavin cofactor, which we have identified as flavin adenine dinucleotide.

ACCESSION NUMBER: 1994:483395 BIOSIS
DOCUMENT NUMBER: PREV199497496395
TITLE: Chemiluminescence immunosorbent assay (CLISA) and a possibility of the specific detection of **soluble** antigens of Clostridium botulinum type A.
AUTHOR(S): Ligieza, Jerzy [Reprint author]; Reiss, Juliusz [Reprint author]; Michalik, Mariusz
CORPORATE SOURCE: Dep. Microbiol., Military Inst. Hygiene and Epidemiol., Kozielska 4, 01-163, Warsaw, Poland
SOURCE: Archivum Immunologiae et Therapiae Experimentalis, (1994) Vol. 42, No. 2, pp. 129-133.
CODEN: AITEAT. ISSN: 0004-069X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 9 Nov 1994
Last Updated on STN: 9 Nov 1994
AB A double antibody version of CLISA was demonstrated to be a rapid method (1 h) for detection and quantitative determination of Clostridium **botulinum toxin** antigens in biological samples. The sensitivity of this assay is about ten-fold higher than both ELISA and passive hemagglutination test. Thus, the double antibody version of CLISA appeared to be useful for the control of food products contaminated with Cl. botulinum type A bacteria.

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ACCESSION NUMBER: 2000:95515 BIOSIS
DOCUMENT NUMBER: PREV200000095515
TITLE: Production and analysis of anti - (**Botulinum toxin**) human antibody fragments.
AUTHOR(S): Quesnel-Hellmann, A. [Reprint author]; Vaivre, R. [Reprint author]; Reynaud, D. [Reprint author]; Michel, P. [Reprint author]; Attree, O. [Reprint author]
CORPORATE SOURCE: C.R.S.S.A., La Tronche, Grenoble, France
SOURCE: Travaux Scientifiques des Chercheurs du Service de Sante des Armees, (1999) Vol. 0, No. 20, pp. 71-72. print.
ISSN: 0243-7473.
DOCUMENT TYPE: Article
LANGUAGE: French
ENTRY DATE: Entered STN: 15 Mar 2000
Last Updated on STN: 3 Jan 2002

AB **Soluble** antibody fragments (scFv) to botulinum neurotoxin type A were produced from clones isolated from a large non-immunized phage display library. scFv production and purification protocols were developed. Among 13 clones assayed, one scFv was partially neutralizing in mice.

L8 ANSWER 43 OF 67 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1946:625 CAPLUS
DOCUMENT NUMBER: 40:625
ORIGINAL REFERENCE NO.: 40:109e-f
TITLE: The nature of Clostridium **botulinum** **toxin**
AUTHOR(S): Rodopulo, A. K.
SOURCE: Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (1941), (No. 10/11), 68-71
CODEN: ZMEIAV; ISSN: 0372-9311
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable

AB The toxin of Cl. botulinum is not a protein, but is mechanically attached to the protein substances of the cell, since it is separated on dialysis. It is an endotoxin and is liberated on destruction of the cell. It is insol. in acetone, Et2O, and alc., difficultly **sol.** in water, and neutralizable by weak alkali.

```
=> T7lac (w) promoter
L1           45 T7LAC (W) PROMOTER

=> solub4
L2      6891262 4

=> L1 and l2
L3           16 L1 AND L2

=> botulinum (l) L16
L16 NOT FOUND
The L-number entered could not be found.  To see the definition
of L-numbers, enter DISPLAY HISTORY at an arrow prompt (=>).

=> Botulinum (l) L3
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'BOTULINUM (L) L7'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'BOTULINUM (L) L8'
L4            0 BOTULINUM (L) L3

=> D L3 IBIB ABS 1-16
```

Example 30

2/13/3

```
=> L8 and Ecoli
L11           0 L8 AND ECOLI

=> "E Coli"
L12      176850 "E COLI"

=> L8 and L12
L13           0 L8 AND L12

=> D L8 IBIB TI 1-64
```

```
=> botulinum (w) toxin (l) L1
L5            0 BOTULINUM (W) TOXIN (L) L1

=> botulinum (w) toxin (L) BL21
L6            0 BOTULINUM (W) TOXIN (L) BL21

=> botulinum (w) toxin
L7         5214 BOTULINUM (W) TOXIN

=> soluble and L7
L8           67 SOLUBLE AND L7

=> L8 and L1
L9            0 L8 AND L1

=> promoter and L8
L10           1 PROMOTER AND L8

=> D L10 IBIB ABS
```

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=> T7lac (w) promoter
L1 45 T7LAC (W) PROMOTER

=> solub4
L2 6891262 4

=> L1 and l2
L3 16 L1 AND L2

=> botulinum (l) L16
L16 NOT FOUND
The L-number entered could not be found. To see the definition
of L-numbers, enter DISPLAY HISTORY at an arrow prompt (=>).

=> Botulinum (l) L3
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'BOTULINUM (L) L7'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'BOTULINUM (L) L8'
L4 0 BOTULINUM (L) L3

=> D L3 IBIB ABS 1-16

L3 ANSWER 1 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:792432 CAPLUS
DOCUMENT NUMBER: 139:377211
TITLE: Expression Cloning and Biochemical Characterization of a Rhizobium leguminosarum Lipid A 1-Phosphatase
AUTHOR(S): Karbarz, Mark J.; Kalb, Suzanne R.; Cotter, Robert J.; Raetz, Christian R. H.
CORPORATE SOURCE: Department of Biochemistry, Duke University Medical Center, Durham, NC, 27710, USA
SOURCE: Journal of Biological Chemistry (2003), 278(41), 39269-39279
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Lipid A of Rhizobium leguminosarum, a nitrogen-fixing plant endosymbiont, displays several significant structural differences when compared with Escherichia coli. An especially striking feature of R. leguminosarum lipid A is that it lacks both the 1- and **4**'-phosphate groups. Distinct lipid A phosphatases that attack either the 1 or the **4**' positions have previously been identified in exts. of R. leguminosarum and Rhizobium etli but not Sinorhizobium meliloti or E. coli. Here we describe the identification of a hybrid cosmid (pMJK-1) containing a 25-kb R. leguminosarum 3841 DNA insert that directs the overexpression of the lipid A 1-phosphatase. Transfer of pMJK-1 into S. meliloti 1021 results in heterologous expression of 1-phosphatase activity, which is normally absent in exts. of strain 1021, and confers resistance to polymyxin. Sequencing of a 7-kb DNA fragment derived from the insert of pMJK-1 revealed the presence of a lipid phosphatase ortholog (designated LpxE). Expression of lpxE in E. coli behind the **T7lac promoter** results in the appearance of robust 1-phosphatase activity, which is